

Are inner cave communities more stable than entrance communities in Lapa Nova show cave?

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Abstract

Lapa Nova is a dolomitic Brazilian show cave. Invertebrate fauna registered for this cave is quite rich and abundant. During two intensive surveys in 2009, 24,482 invertebrate individuals belonging to 187 species were sampled. We found 160 species in April sampling, while in September sampling richness was considerably lower, 102 species, with a remarkable species turnover. In this paper the species richness, abundance and species diversity is presented. The Shannon's diversity index was 2.79 and 2.87 for April and September, respectively. Although one would expect less variations to be found in the deep cave community (when compared to those located near the entrances), due to higher environmental stability, this was not observed at Lapa Nova cave. This "paradox" is probably due to the intense tourism that occurs in the cave, which imposes "instability" in all sectors, not only in nearby entrance areas. Visitation at the cave probably altered the expected natural distribution pattern, imposing a new organization of the communities, driven by the unstable conditions imposed by cave tourism.

Keywords

Cave invertebrates, temporal turnover, beta diversity, tourism impact, Brazil

Introduction

Despite the well known environmental stability in subterranean systems, it is not homogeneous for the whole extension of a cave. With the use of precise monitoring instruments, certain environmental variability can be detected (Romero 2009). Entrance areas in caves present direct atmospheric exchange with the cave (Oliveira et al. 2008, Lobo et al. 2009, Lobo et al. 2015, Tobin et al. 2013). Such areas can be considered as ecotones, situated in boundaries between two relatively homogeneous ecological communities, hypogean and epigean (Prous et al. 2004, Moseley 2009, Prous et al. 2015). Variability in parameters such as temperature is attenuated with increasing distance from the cave entrance (Tobin et al. 2013). Moreover, differences among transitional areas of the caves tend to be more drastic in caves situated in temperate areas (Tobin et al. 2013), when compared to tropical areas.

In spite of the differences in stability among caves situated at different latitudes, the range of environmental instability intensity can also vary in different caves located in a same region. Such differences occur according to their physical conditions, presence of large bat populations, human use, among others (Huppop 2005, Rocha 2013, Lobo et al. 2015). Cave morphology, entrance size and the number of entrances, are important factors influencing stability (Ferreira 2004, Tobin et al 2013). In some cases, larger entrances usually lead to an increase in temperature and humidity seasonal variation, since they impose a more ample connection with the epigean environment (Ferreira 2004, Tobin et al 2013). Accordingly, one would expect less variation to be found in the inner communities when compared to those located near the entrances, due to higher environmental stability. Furthermore, subterranean rivers can also enhance the atmospheric instability inside caves, amplifying the connectivity with the outside (Lobo et al. 2015) and the climatic variations inside caves. In the tropics, huge movements of bat populations leaving and returning to the cave can also cause microclimate changes in subterranean environment (Rocha 2013). Such differences are also detected along sectors in the same cave; daily bat movements are reflected in cave chambers (Rocha 2013). Seasonal stability differences are also reflected in the distribution and abundance patterns of populations associated to the cave environment (Romero 2009). Therefore, seasonal changes exert a considerable influence on cave fauna distribution; many troglobionts and troglophiles are restricted to areas with the most stable temperature and humidity (Tobin et al. 2013) like the deeper passages.

Factors other than microclimate, can determine changes in the distributional patterns of subterranean species. Human use is an important factor that generates instability in the cave environment, especially in cases of high intense tourism. This type of use can modify patterns such as temperature, humidity and speleothem growth (Lobo 2015), and modify distribution patterns of populations inside subterranean environments (e.g. Moldovan et al. 2003; Bernard et al. 2010; Pellegrini and Ferreira 2012; Guil and Trajano 2013, Faille et al. 2015). As an example, high impacts due to intense tourism were detected in Ursilor Cave (Transylvania, Romania), which demonstrated drastic reduction of two endemic cave beetles in sectors with visitation (Moldovan et

al. 2003). The monitoring of their populations conducted before and after tourism implementation showed that such species have altered their distribution and abundance in Ursilor Cave in response to visitation disturbing events. Such species became almost absent in traps installed in the touristic region of the cave, showing that cave visitation is an intensive disturbance for those populations.

Studies investigating distribution of cave fauna have only focused on the cavernicolous species (troglobites) (e.g. Moldovan et al 2003). Troglobites are more frequently found in deeper and more isolated cave areas, with greater environmental stability (Peck 1976, Tobin et al 2013), and in areas of lower impact degree caused by tourists (Moldovan et al 2003, Pellegrini and Ferreira 2012). Although Novak et al. (2012) indicated that terrestrial troglobite fauna is more diverse and randomly distributed in entrance areas (corresponding to the first 10 meters of the cave) and in the shallow subterranean habitat.

Although a recent study indicates the existence of two hotspots of subterranean biodiversity in Brazil (Souza-Silva and Ferreira 2016), Neotropical cave communities are formed mainly by troglophilic organisms (Trajano and Bichuette 2010). In many Brazilian caves, troglobitic species are even unknown (Souza-Silva et al. 2011, Simões et al. 2014, Simões et al. 2015). Thus, studies in Neotropical caves focusing only on troglobite species would eventually neglect most of the species that make up the entire cave community.

However, studies that have accessed temporal and spatial variations of the entire subterranean community are scarce. The understanding of seasonal patterns generates subsidies for subterranean ecosystem conservation and management purposes. As such, the objective of the present work was to identify the alterations undergone by the invertebrate community associated to Lapa Nova, a large dolomitic cave of Minas Gerais state, Brazil, in two different sampling periods, considering two main “compartments”: inner communities and those communities associated to areas near entrances. Our main intention was to verify if inner communities are more “stable” than entrance communities considering the mean richness and abundance values in time, and also temporal beta diversity values and species composition variation, which, in theory, are more subject to external variations. It worth stating that in the publication by Pellegrini and Ferreira 2012, changes undergone by the community in an interval of two consecutive months (April and May) due to the cave tourist use were assessed, with a subsequent proposal of an appropriate management plan for the cave. Unlike that study, this present study focuses on community stability in a period of five months, comprising two distinct seasons.

Materials and methods

Study area

The present study was conducted at the Lapa Nova dolomitic show cave, located in Vazante, northwest Minas Gerais state, Brazil (Fig. 2A) ($17^{\circ}59'04.0''S$ $46^{\circ}53'26.4''W$).

It is the most known and visited cave in northeastern Minas Gerais state, besides being the second most extensive cave of the area with 4,550 meters of linear development (Auler et al. 2001). It is a hypogenic, labyrinthine cave, network shaped, with one big downward entrance and two secondary entrances (Auler et al. 2009).

In order to confirm if the sectors near entrances are more variable when compared to more isolated sectors, we used humidity and temperature data. This data is available at Lapa Nova Management Plan, and it was measured during four days in April (Auler et al. 2009), in the same year that this work was conducted. The external sampling site (TH1), near the principal cave entrance, showed the highest range of temperature and air relative humidity variation from 17.9 to 20.5°C and from 91 to 99% respectively (Figure 1). The entrance-sampling site (TH2) presented a variation from 17.6 to 19.3°C and from 97 to 99%. The two sampling sites in deeper regions (TH3 and TH4) of the cave were considerable stable. One site presented a variation from 18.8 to 19.3°C and air humidity was constant at 99% and the other from 18.9 to 19.8°C and from 97 to 99% (Auler et al. 2009).

Invertebrate sampling

The cave was divided into nine sectors, each corresponding to 1/9 of the total linear extension of Lapa Nova. Three sectors were located in entrance areas (Sectors 1, 4 and 5), the other six were in deeper regions of the cave (Sectors 2, 3, 6, 7, 8 and 9) (Figure 1).

Two five-day field trips were carried out for collecting in the nine sectors, the first was in April, and the other in September, both in 2009. The invertebrate collections were conducted by the same team, composed of five biologists with experience in caving and invertebrates collection, and it was done through manual capture (with the aid of tweezers, brushes and hand nets). Sampling was conducted by visual searching throughout all the accessible places in the cave, prioritizing organic deposits (debris, carcasses, guano, etc.) and microhabitats (spaces under rocks, humid soil, cracks, speleothems, etc.). All the invertebrate species found in the sectors had some of their specimens collected. The organisms observed during the collections were counted and plotted on the cave map according to the methodology proposed by Ferreira (2004), allowing a visualization of the distribution, as well as the relative abundance of the different species found in the cave.

All the collected invertebrates were identified to the lowest taxonomic level possible, using a stereomicroscope. The specimens were separated into morpho-species for determination of species richness. The collected specimens were fixed in 70% alcohol. Subsequently they were deposited in the *Centro de Estudos em Biologia Subterrânea* collection (Zoology Sector / Biology Department, Federal University of Lavras). Troglobiotic species were considered as troglobite, we performed visual searching outside the cave looking for those species under rocks and wood debris and they were not found, indicating that they are restricted to the subterranean environment.

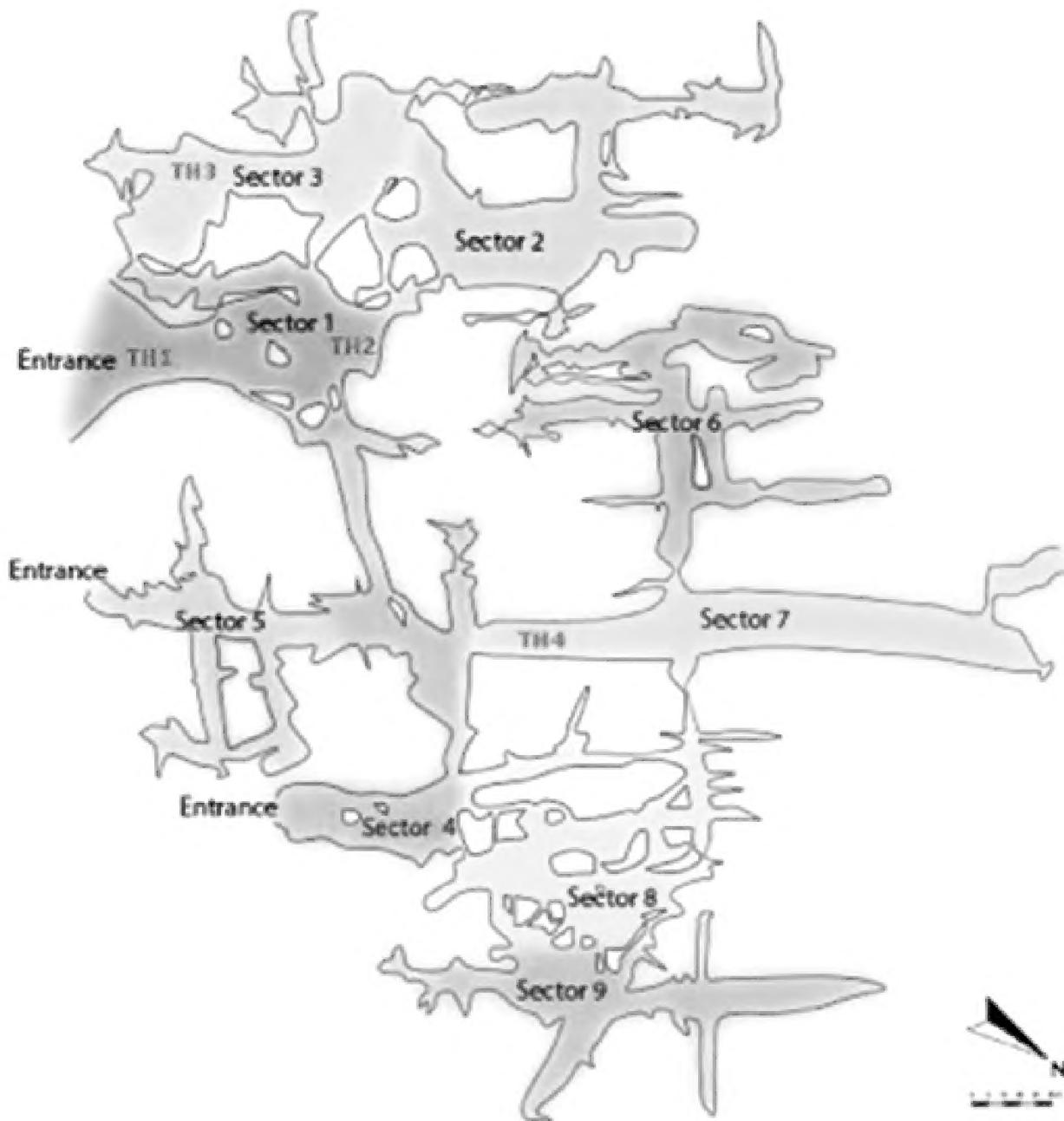


Figure 1. Lapa Nova Show cave map depicting the sectors: green tones correspond to entrance areas; the other six colors correspond to deeper regions of the cave. Legend: (TH) Temperature measurement areas.

Data analysis

In each sector we determined the richness, abundance and diversity of the invertebrate communities for each sampling period. The diversity calculation was made using the Shannon-Wiener index (Magurran 1988). In order to compare three entrance sectors with six inner sectors, we calculated mean richness and mean abundances values.

Accessing beta diversity components allow inferring about processes driving species distributions and biodiversity (Baselga and Orme 2012). Beta diversity components can result from species replacement (*turnover*) or species loss / gain (nestedness) (Baselga and Orme 2012). In order to determine temporal species composition differences, considering sectors between both sampling events, beta-partitioning diversity was calculated. For accessing temporal beta diversity, and also turnover and nestedness contribution for

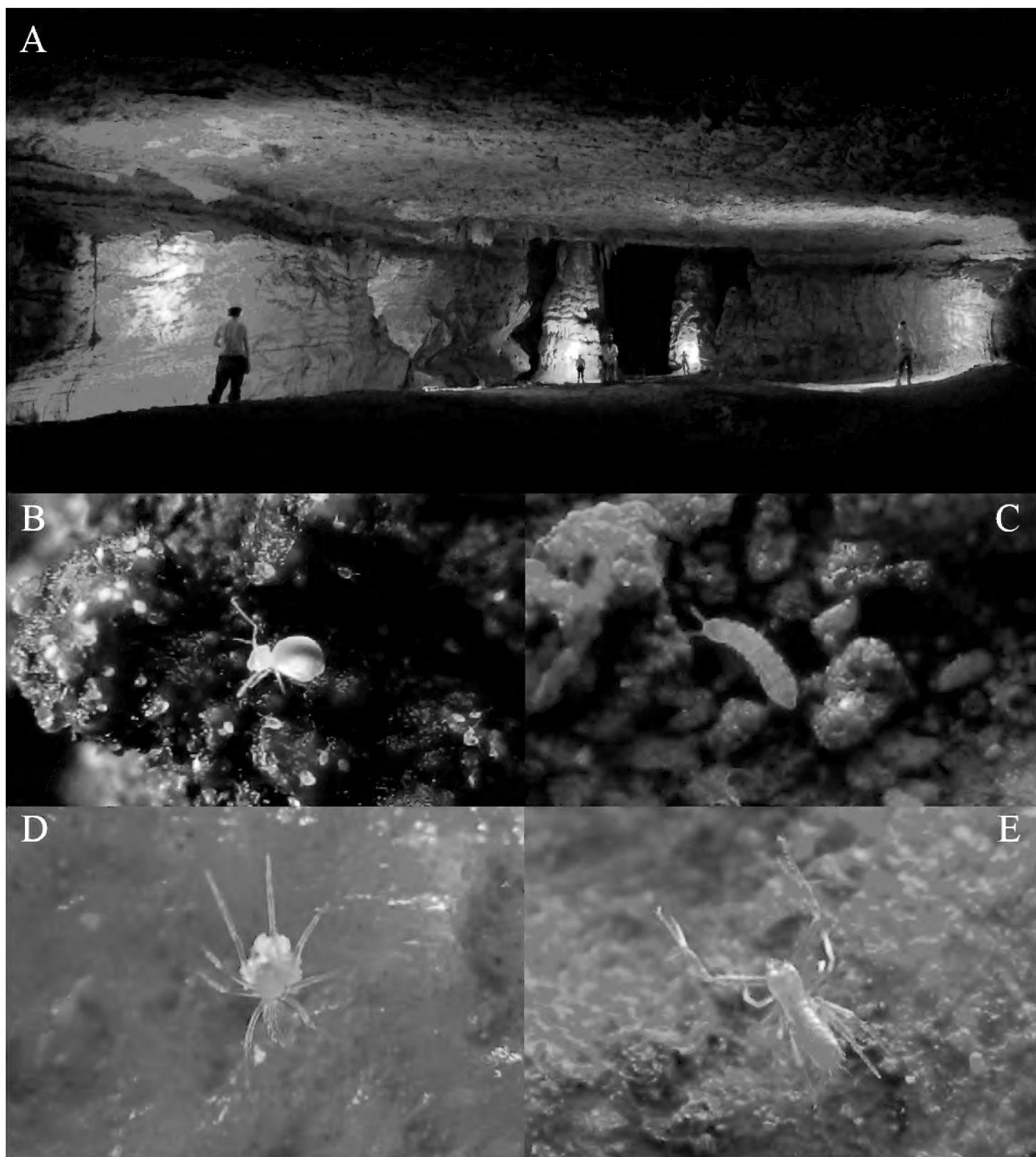


Figure 2. **A** Passage in Lapa Nova Cave **B, C, D, E** Examples of troglomorphic species recorded in the Northwest region of the state of Minas Gerais, Brazil. **B** Collembola: Arrhopalitidae: *Arrhopalites* sp. 1 **C** Collembola: Poduromorpha, *Acherontides* sp. 1 **D** Araneae: Ochyroceratidae sp. 1 and **E** Pseudoscorpiones: Chthoniidae, *Pseudochthonius* sp. 1.

total diversity, the BETA.TEMP function from the BETAPART package was used. According to Baselga and Orme (2012), this analysis compares two presence-absence species matrices from the sectors, at two different sampling events (April and September), and computes pairwise turnover components over time within each sampled sector. Total diversity was then computed by the Sorensen or Jaccard dissimilarity index. Finally, differences among beta diversity components, between entrances and inner sectors, were tested using ANOVA test, function AOV, from the STAT package. All analyses were

performed in R version 3.2.4 (R Development Core Team, 2014). In this work, we assumed that more stable sectors of the cave would present lower beta diversity values, reflecting lower species replacement or lost/gain rates, when compared to unstable sectors.

The similarity was evaluated among the fauna of all of the sectors of the cave, in the two periods. For that we used the Non-metric Multidimensional Scaling (n-MDS). The n-MDS was built based on the quantitative composition of the invertebrate fauna using the Bray-Curtis index. The existence of significant differences of the groups generated by the n-MDS was evaluated through ANOSIM, also done by the Bray-Curtis index. Finally, the SIMPER analysis was used to evaluate which species were responsible for such differences. All of the above analyses were conducted through the PRIMER 6.0 program.

Results

In Lapa Nova, a total of 24,482 individuals were recorded distributed in 187 species. From this total, 16,996 were registered in the April sampling, and 7,486 in the September sampling. The richness found in April was 160 species, while in September, that number was lower, 102 species. Of the total of 187 species, 85 were only observed in the first sampling event, 26 only in the second and 76 occurred in both periods. Diversity values were 2.79 and 2.87 for the first and second sampling events, respectively.

In April, Diptera was the order that presented the highest richness, with 33 species, followed by Araneae and Coleoptera with 31 and 29 species respectively. In September, the richness was much lower, the highest values being presented by Diptera and Araneae, both with 15 species, followed by Coleoptera with 12 species (Table 1).

Six troglomorphic species were found: *Arrhopalites* sp. (Collembola: Arrhopalitidae) (Fig. 2B), *Acherontides* sp. (Collembola: Hypogastruridae) (Fig. 2C), *Eukoenenia virgemdalapa* Souza and Ferreira, 2012 (Palpigradi: Eukoeneniidae), one Oonopidae (Araneae), one Chthoniidae (Pseudoscorpiones) (Fig. 2E) and one Stylopiscidae (Isopoda) (Figure 2). Such troglomorphic species, did not present many differences in abundance and richness values among entrances and deeper sectors (Table 1).

The sectors of the cave did not present significant differences when comparing the two sampling events. There was a large overlap of species abundance in both sampling events by the similarity analysis conducted through n-MDS. Similarly, the ANOSIM test between the two periods was not significant ($p=0.062$) (Figure 3).

The average richness in entrance sectors and inner sectors was quite distinct. In entrance areas the average richness corresponded to 66 species in the first sampling event and 54 species in the second. Inner areas of the cave present an average of 28 species in April and 24 species in September (Table 2). The mean “turnover” of species was found to be 46.66 in entrance sectors, 44.72 in sectors at the mediations of entrance areas and 47.83 in inner sectors (Table 2). No pattern in differences in total beta diversity or in beta diversity components was found among inner or entrance sectors by the ANOVA test.

Table 1. Invertebrates collected and their abundance in the different sections in both seasons.

	SECTORS IN APRIL SAMPLING									SECTORS IN SEPTEMBER SAMPLING								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
NEMATODA																		
Nematoda sp.1	11		13							50								
OLIGOCHAETA																		
Lumbricidae sp.1	42									1			2					
HIRUDINEA																		
Hirudinida sp.1										1								
PULMONATA																		
Styliommatophora sp.1		1																
Styliommatophora sp.2			2															
Styliommatophora sp.3			3															
Styliommatophora sp.4										1								
SARCOPTIFORMES																		
Acaridae sp.1	50																	
Astigmata sp.1										1								
Oribatida sp.1										1								
TROMBIDIIFORMES																		
Anystidae (<i>Erythracarus</i> sp.1)	1		19							6			2					
Bdellidae (<i>Bdellodes</i> sp.1)	3									1								
Cheyletidae sp.1	1												6					
Rhagidiidae sp.1	5		1	9						1			14					
Teneriffidae (<i>Neovenefifolia xerophila</i>)										11								
IXODIDA																		
Ixodidae sp. 1	1																	
MESOSTIGMATA																		
Laelapidae sp. 1										1								
Laelapidae (<i>Stratiolaelaps</i> sp.1)										1			2					
Macronyssidae sp.										2			2				2	

	SECTORS IN APRIL SAMPLING			SECTORS IN SEPTEMBER SAMPLING		
	1	2	8	1	2	9
Melicharidae (<i>Proctolaelaps</i> sp.1)						
Ologamasidae sp.1	2	1				
Podocinidae (<i>Podocimum</i> sp.1)				8		
ARANEAE						
Araneae sp.1		1				
Araneae sp.2	1					
Araneae sp.3			2			
Ctenidae (<i>Isoctenus</i> sp.1)	320	2	11	6	12	20
Deinopidae sp.1			1		4	1
Gnaphosidae sp.1	1				22	12
Sicariidae (<i>Loxosceles variegata</i>)	71	8	8	180	72	34
Sicariidae (<i>Loxosceles</i> sp.2)		1			855	932
Nemesidae sp.1					115	3
Nemesidae sp.2	3				12	144
Caponiidae (<i>Nops</i> sp.1)				1	49	29
Onopidae sp.1		4		1	29	139
Pholcidae sp.1			2	5	4	995
Salticidae sp.1					1	917
Salticidae sp.1	11			1		
Segestriidae sp.1				1		
Theridiidae sp.1		6				
Theridiidae sp.10	19	2	25	3	8	2
Theridiidae sp.11				2		
Theridiidae sp.12				1		
Theridiidae sp.13					2	
Theridiidae sp.14						1
Theridiidae sp.2				1		
Theridiidae sp.3				1		
Theridiidae sp.4	1			1		
Theridiidae sp.5				1		1

	SECTORS IN APRIL SAMPLING				SECTORS IN SEPTEMBER SAMPLING			
	1	2	3	4	1	2	3	4
Theridiidae sp.6								
Theridiidae sp.7		2						
Theridiidae sp.8		1	1		1			
Theridiidae sp.9	1	11	1	4		18	3	5
Theridiidae (<i>Theridium</i> sp.1)	10	11	18	7	3	21	2	7
PALPIGRADI								
Eukoeniidae (<i>Eukoenia</i> sp.1)	9				3	31	1	4
Eukoeniidae (<i>Eukoenia virgemdalapa</i>)							1	
PSEUDOSCORPIONES								
Cheiridiidae sp.1	20				5	2	2	6
Chernetidae sp.1	26				3	15	4	
Chernetidae sp.2					1		7	1
Chthoniidae sp.1	3	3	2		1	1	2	1
OPILIONES								
Gonyleptidae sp.1	3					2	2	
Gonyleptidae sp.2	1					4		
Gonyleptidae sp.3	5	8	25		2	8	4	
Opiliones sp.1 (juvenile)			3			1	2	4
ISOPODA								
Dubioniscidae sp.1					3		1	1
Sytoniscidae sp.1							7	2
Plathyarthridae (<i>Trichorhina</i> sp.1)	10						6	1
OSTRACODA								
Ostracoda sp.1	21							35
Ostracoda sp.2								
CHILOPODA								
Geophilidae sp.1							1	
Henicopidae sp.1	7							2
Scutigeromorpha sp.1	1		1	11			1	

DIPLOPODA	SECTORS IN APRIL SAMPLING			SECTORS IN SEPTEMBER SAMPLING		
	1	2	3	1	2	3
COLLEMBOLA						
<i>Hypogastruridae (Acheronitidae) sp.1)</i>	1			1	6	1
<i>Arrhopalitidae (Arrhopalites) sp.1)</i>	4	2		10	3	1
<i>Collembola</i> sp.1	19	12	4	3	1	1
<i>Collembola</i> sp.2	184	1	40	12	14	14
<i>Collembola</i> sp.3	2		3	23	36	2
<i>Collembola</i> sp.4				3	31	13
<i>Collembola</i> sp.5					3	1
BLATTODEA					19	1
<i>Blattodea</i> sp.1	23	4	9	29	11	49
<i>Blattodea</i> sp.2			1			
COLEOPTERA						
<i>Carabidae</i> sp.1	9				15	3
<i>Carabidae</i> sp.2	16					
<i>Carabidae</i> sp.3					1	
<i>Cholevidae</i> sp.1	266		6	85	21	3
<i>Elateridae</i> sp.1					1	
<i>Elateridae</i> sp.2						
<i>Histeridae</i> sp.1					3	
<i>Carabidae</i> (Larvae)	4					
<i>Coleoptera</i> sp.10 (Larvae)				1		
<i>Coleoptera</i> sp.12 (Larvae)					1	
<i>Coleoptera</i> sp.8 (Larvae)					1	
<i>Dermestidae</i> sp.1 (Larvae)					1	
<i>Dermestidae</i> sp.2 (Larvae)					1	
<i>Staphylinidae</i> sp.2 (Larvae)	8					
<i>Staphylinidae</i> sp.6 (Larvae)	200					
<i>Tenebrionidae</i> sp.1 (Larvae)					1	1

	SECTORS IN APRIL SAMPLING						SECTORS IN SEPTEMBER SAMPLING					
	1	1	1	1	1	1	3	3	3	5	2	13
Tenebrionidae sp.2 (Larvae)												
Ptiliidae sp.1							1	1	1			
Ptilodactylidae sp.1		22			4	1	2	2	11	1		3
Staphylinidae sp.5			7	1	9			2		1		
Staphylinidae sp.1						1						
Staphylinidae sp.2												
Staphylinidae sp.3			5		2						3	
Staphylinidae sp.4		28			19		1				3	
Staphylinidae sp.6				1							3	
Tenebrionidae (<i>Zophobas</i> sp.1)	1		8		10		2		4		1	1
DIPTERA												
Cecidomyiidae sp.1						1			1			
Conicera sp.1	5		2		7	3		52	61	47	9	16
Diptera sp.1								7	11	3	3	3
Diptera sp.2			1									
Diptera sp.4				1				1				
Diptera sp.5					1	2						
<i>Drosophila</i> sp.1	13			6	1	2	8		2003	7	1	1
Drosophilidae sp.2								50		1		
Chironomidae sp.1 (Larvae)	1					1						
Chironomidae sp.2 (Larvae)	19	10										
Chironomidae sp.3 (Larvae)	2	1						3		4		
Diptera sp.1 (Larvae)	61	100	3		16	25	2	2550		125		50
Diptera sp.2 (Larvae)	61	100	1		16	25	1	2250		125		50
Diptera sp.3 (Larvae)	8									3		
Diptera sp.4 (Larvae)										1		
Diptera sp.5 (Larvae)										1		
Keroplatidae sp.1 (Larvae)	1	1										
Mycetophilidae sp.1 (Larvae)											2	
Milichiidae sp.1								16				

	SECTORS IN APRIL SAMPLING						SECTORS IN SEPTEMBER SAMPLING					
	1	2	3	4	5	6	1	2	3	4	5	6
Hymenoptera sp.1						6						
Hymenoptera sp.3					1							
Formicidae (<i>Acromyrmex</i> sp.1)				4			1					
Formicidae sp.1					3							57
Formicidae sp.2	1											
Formicidae sp.3	1			2								
Formicidae sp.4				27	5	6		8				7
ISOPTERA												
Nasutitermitinae sp.1						3						1
LEPIDOPTERA												1
Arctiidae sp.1							1					
Noctuidae (<i>Hypena</i> sp.1)	21	3	98	9	12	82	75	1	131	19	187	35
Lepidoptera sp.1 (Larvae)								1				
Tineidae sp.1 (Larvae)	5	10	38	6	40	17	104	22	342	58	1	3
Tineidae sp.2 (Larvae)											1	19
Erebidae (<i>Latebraria</i> sp.1)				1					2	1		
Lepidoptera sp.1 (Pupae)		1								2		4
Tineidae sp.1	2			1			1			5		
Tineidae sp.2	5	1	3	7	4	13	24	228	14	2	1	12
PSOCOPTERA												6
Ptiloneuridae (<i>Euplocania</i> sp.1)	6											
Ptiloneuridae (<i>Euplocania</i> sp.2)	6											
Lepidopsocidae (<i>Nepticulomima</i> sp.1)	8								3			5
Psocoptera juvenile sp.4	3									5		
Psocoptera juvenile sp. 5	8									11		9
Psyllipsocidae (<i>Psyllipsocus</i> sp.1)	8	14	9	5	21		15	81	7	49	64	5
Psyllipsocidae (<i>Psyllipsocus falcifer</i>)	14	24	15	2	7	35	24	137	11	83	109	8
Psyllipsocidae (<i>Psyllipsocus</i> sp.3)	1		2	1		3	3	14	1	8	11	5
Psyllipsocidae (<i>Psyllipsocus</i> sp.4)								3		3	20	5

Table 2. Richness and Diversity values of the ten sectors in both sampling events.

Sector	April Richness	September Richness	Total Richness	Mean Richness	Turnover	Nestedness	β-Diversity
1 (entrance)	78	91	122	84.5	35.53	5.79	41.32
2 (inner)	29	23	38	26	39.13	7.02	46.15
3 (inner)	26	21	33	23.5	33.33	7.09	40.42
4 (entrance)	56	60	85	58	44.64	1.90	46.55
5 (entrance)	27	44	54	35.5	37.04	15.08	52.11
6 (inner)	30	38	47	34	30.00	8.23	38.24
7 (inner)	26	31	40	28.5	34.62	5.73	40.35
8 (inner)	29	37	50	33	44.83	6.69	51.52
9 (inner)	24	38	47	31	37.50	14.11	51.61

Table 3. SIMPER analysis. Species that mostly contributed to dissimilarity presented by entrance sectors in both sampling events.

Taxa	Individual Contribution	Cumulative %	September	April
<i>Pericoma</i> sp.1	24.59	24.59	30	762
<i>Hypena</i> sp.1	7.08	31.67	118	42.7
<i>Loxosceles variegata</i>	5.54	37.21	103	108
<i>Psyllipsocus falcifer</i>	5.34	42.55	84.7	10.3
<i>Collembola</i> sp.2	4.35	46.90	56.3	78.7
<i>Isoctenus</i> sp.1	3.64	50.54	18	112
<i>Psyllipsocus</i> sp.1	3.21	53.75	50	5.67
<i>Cholevidae</i> sp.1	3.02	56.77	7.33	90.7
<i>Tineidae</i> sp.1	2.62	59.39	19.3	17
<i>Conicera</i> sp.1	2.53	61.92	36	2.33
<i>Theridiidae</i> sp.10	2.17	64.08	33.7	8
<i>Staphylinidae</i> sp.6	1.95	66.04	0	66.7
<i>Endecous</i> sp.1	1.87	67.91	18.7	46.7
<i>Eidimanacris</i> sp.1	1.57	69.48	24	10.3

Entrance sectors and inner sectors also did not present significant differences among species composition and relative abundance between both sampling events (Figure 4), for the ANOSIM test through the Bray-Curtis index ($p > 0.40$). On the other hand, it was possible to observe a considerable variation in composition when observing each sector. The highest variation observed between the two sampling events was observed in sector one, at an entrance area, but also in sector nine, in a deep area of the cave.

The species that mostly contributed to the dissimilarity observed in the entrance sectors between both sampling events were: *Pericoma* sp. (Psychodidae, Diptera), responsible for approximately 24.5% of such differences; followed by *Hypena* sp., responsible for 7%; *Loxosceles variegata* (Sicariidae, Araneae), with 5.5%; *Psyllipsocus falcifer* (Psocoptera), with 5.3%; *Collembola* sp.2, with 4.3% and *Isoctenus* sp (Ctenidae, Araneae) 3.6%

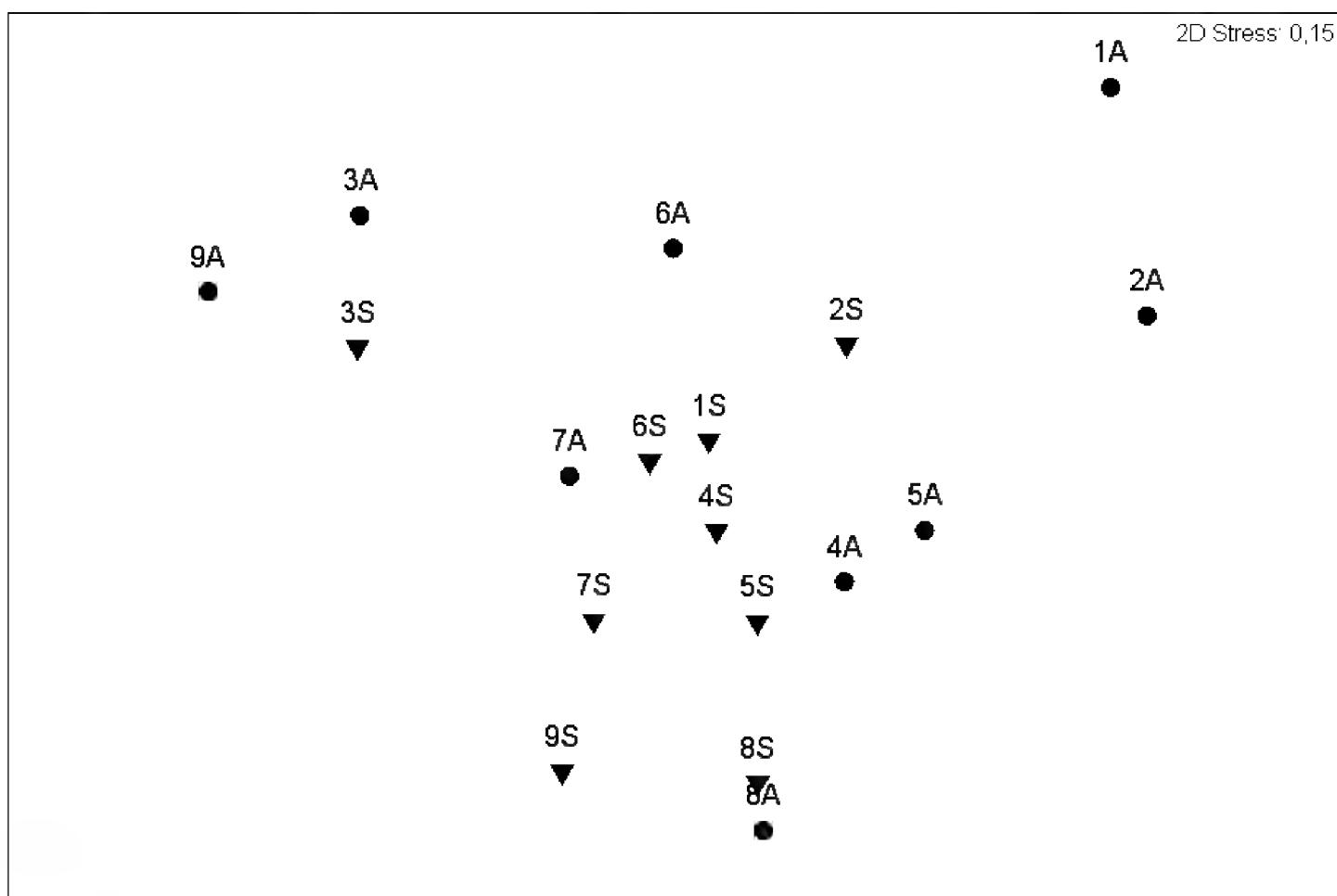


Figure 3. Multidimensional scaling (n-MDS) of sectors collected in April (circles) and September (triangles) periods, by the Bray-Curtis quantitative similarity index.

(Table 3). These species, together, accounted for 50% of the dissimilarities presented between the entrance sectors in both periods.

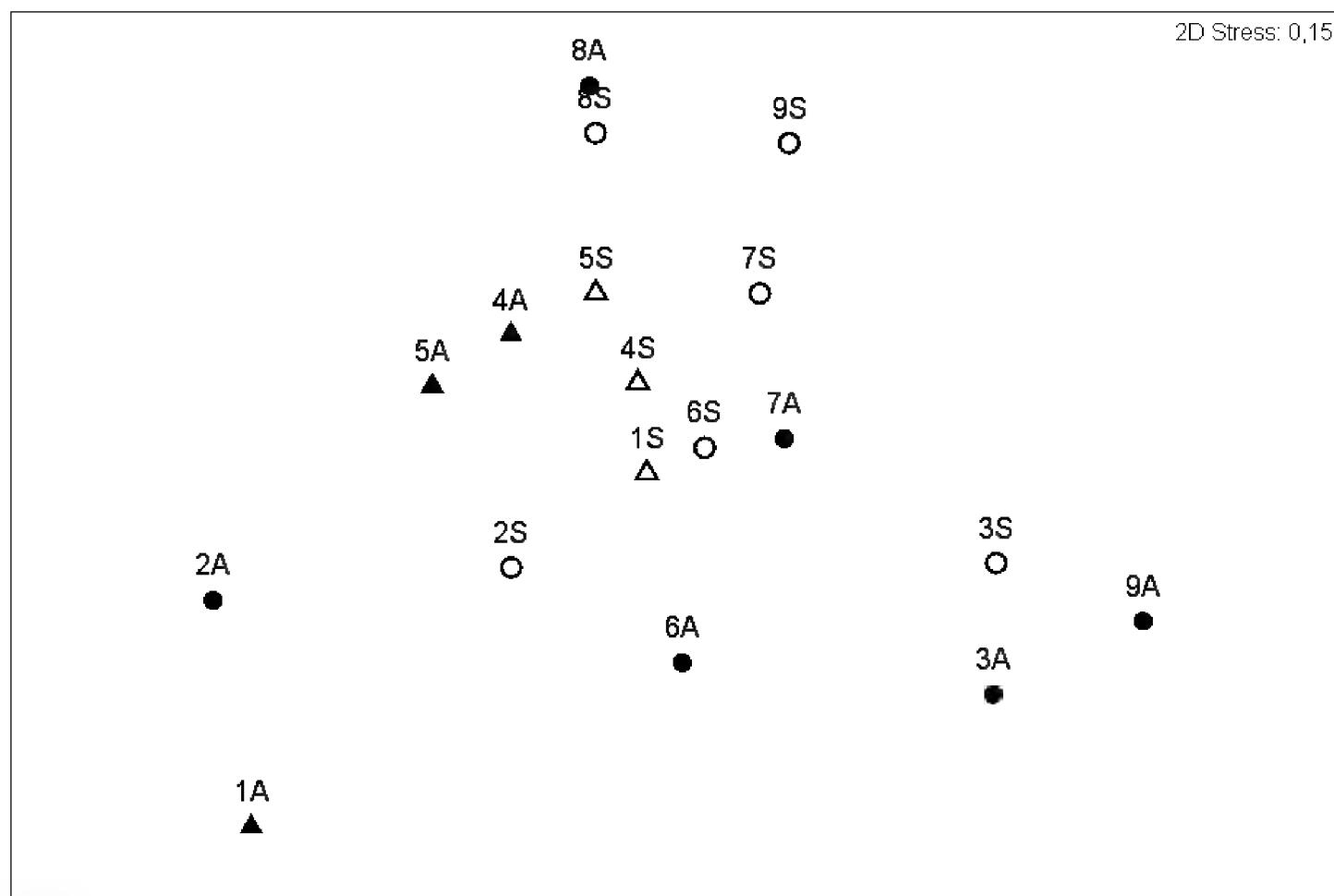
Deeper regions of the cave presented a different pattern. The species that most contributed to differences presented by such sectors were *Loxosceles variegata*, responsible for approximately 25.5% of such differences; followed by two species of dipteran larvae that together are responsible for 20% and *Psyllipsocus falcifer* (7.5%). Those species, together, were responsible for more than 50% of the similarities among the inner sectors in both periods (Table 4). None of the real cave species (troglobites) presented significant differences among sectors when considering both sampling events.

Discussion

The most common species found in Lapa Nova were those most ubiquitous in north-western Minas Gerais limestone caves (Simões et al. 2014). The proportion of troglo-morphic species in Lapa Nova (3.2% of the total) was above the average found for carbonatic caves present in the Brazilian Atlantic Forest (2.41%), and also for the proportion found in the Arcos-Pains-Doresópolis province, which is currently considered the area with the highest concentration of caves in Brazil (1.66%) (Zampaulo 2010, Souza-Silva et al. 2011). It worth pointing out that 72.34% of the caves sampled

Table 4. SIMPER analysis. Species that mostly contributed to dissimilarity presented by deep sectors in both sampling events.

Taxa	Individual Contribution	Cumulative %	September	April
<i>Loxosceles variegata</i>	25.56	25.56	349.17	315.67
Diptera sp.1	10.25	35.81	29.50	448.83
Diptera sp.2	9.55	45.35	29.67	398.67
<i>Psyllipsocus falcifer</i>	7.59	52.94	98.33	15.00
<i>Drosophila</i> sp.1	4.94	57.88	2.67	335.50
<i>Psyllipsocus</i> sp.1	4.48	62.36	58.33	9.17
Tineidae sp.1 (Larvae)	4.21	66.57	4.33	88.83
<i>Endecous</i> sp.1	3.51	70.08	41.17	63.17

**Figure 4.** Multidimensional scaling (n-MDS) of sectors in entrance areas in April (A-black triangles), and sectors in deep areas of the cave in April (A-black circles), and sectors in entrance areas in September (S-white triangles), and sectors in deep areas of the cave in September (S-white circles), by the Bray-Curtis quantitative similarity index.

in northwestern Minas Gerais present only one or no troglobitic species, while only 4.26% of the caves possess more than six troglobitic species (Simões et al. 2014). Accordingly, in a regional context, Lapa Nova Cave can be considered as an extremely important cave regarding endemic cavernicolous species with 6 species, being considered as a priority cave for conservation in the region (Simões et al. 2014).

Temporal differences in species composition

Despite the common statement that caves are stable environments, such environments can have strong responses to changing surface climate (Tobin et al. 2013), and also to changes inside caves (Rocha 2013). Studies concerning cave community variations related to the sampling period concentrate on temperate areas, where those variations are more striking, with an important impact on the fauna (Romero 1983, 2002, 2009, Tobin et al. 2013). In Brazilian caves, there is an indication of a less pronounced variation, though it is, in many caves, quite noticeable (Gomes et al. 2000). While some populations underwent more drastic alterations, others remained with their abundance and spatial distribution little changed (Gomes et al. 2000, Ferreira et al. 2015). The same was observed at Lapa Nova Cave, where some species had a severe reduction in the number of specimens visible in the cave, while others showed an increase in abundance. Some species presented only small abundance alterations, as in the case of the brown spider (*Loxosceles variegata* Simon, 1897). It is important to mention that this is a troglophilic species, which is prone to tolerate higher environmental variations, especially when compared to troglobitic species (Trajano and Bchuette 2010). However, such species showed variation in individual distribution along cave sectors. This dispersion pattern presented by brown spiders among cave sectors was already demonstrated in Lavoura Cave (Minas Gerais state, Brazil), in which some specimens migrated more than 80 meters during a month (Ferreira et al. 2005). The authors attributed such movement to an irregular food resource distribution within the cave. Therefore, spiders had to travel longer distances searching for prey.

Considering other biological parameters such as richness and diversity: richness usually increases in the rainy period; diversity, in turn, does not show a well-established pattern (Ferreira 2004). At Lapa Nova Cave, richness and abundance increased in April, while diversity was higher in September. Richness and abundance variation between two sampled periods can also reflect reproductive periods of some species (Pellegatti-Franco 2004). Moreover, studies indicate that the morphology of the cave walls and the climatic conditions regulate the distribution of moth species within a cave ecosystem (Bourne 1976). Furthermore, the reduction of percolation water availability should favor a wider dispersion of the organisms throughout the cave, in search of more favorable microclimatic conditions. During the September field sampling, the drier sampling event, it was observed *in loco* that organisms were concentrated in areas where there were patches of moisture on the soil, like small pools on areas. These areas were distributed in scattered points in the cave, thus favoring a wider dispersion of the organisms.

Spatial distribution patterns

Cave and subterranean mine entrance areas present a greater density of many populations (Culver and Poulson 1970, Peck 1976), and also higher richness, especially when

compared with inner portions of the caves (Prous et al. 2004, 2015). The same was observed at Lapa Nova Cave, where the entrance areas presented a richness average higher than those observed in the inner areas of the cave. Considering cave entrances as ecotones, the high richness in such transitional zone communities is certainly expected, since these areas present species from both adjacent environments plus exclusive species (Prous et al. 2015).

Beyond the higher richness in entrance areas, one would expect less variation to be found in the inner communities when compared to those located near the entrances, due to higher environmental stability. However, at the Lapa Nova cave such tendency was not observed. Despite the higher climatic differences at entrances areas, inner communities did not vary less, in composition, than those located near entrances, as observed through temporal beta diversity. The high species turnover in all sampled sectors reflects species replacements (Baselga and Orme 2012), due to cave community instabilities. This “paradox” observed in Lapa Nova Cave is probably related to a singular condition of this cave. This cave experiences intense tourism, concentrated over a single period of the year, during a religious festival in honor of the Virgem da Lapa (“Virgin of the Cave”), which occurs early in the month of May. This intense tourism has severe impacts on invertebrate fauna, as well as changes in the spatial distributions of the community (Pellegrini and Ferreira 2012). Tourism is more intense at the main entrance of the cave. This area corresponds to Sector 1, that is one of the most variables sectors of the cave considering the similarity between the two periods analyzed. Therefore, the fauna moves to inner portions of the cave (Pellegrini and Ferreira 2012), generating a huge population displacement into the entire cavity. Tourism also influences microbial distribution. It causes conidial transfer from different sectors in the cave or even allochthonous import from the epigean system (Taylor et al. 2013). In this sense, tourism causes instability in all sectors of the cave, since it is not restricted to entrance areas.

Tourism instabilities

The touristic use of a cave, if uncontrolled, can potentially lead to population size reduction of some species, and this is especially dramatic considering troglobitic species. At Areias Cave System in Brazil, intense tourism led to a strong reduction in the population size of the cavefish, *Pimelodella kronei* (Guil and Trajano 2013). The same observation occurred in a study conducted at Ursilor Cave (Romania) before and after its opening as a show cave (Moldovan et al. 2003). On the other hand, other studies have shown obligate cave species (majority represented by arachnids, crustaceans and beetles) co-existing with cave tourism, as observed in many show caves around the world, such as the Lapa Nova Cave in Brazil, Mammoth Cave, Kentucky, System Postojna-Planina and Krizna Jama Cave in Slovenia, Vjetrenica Jama Cave in Bosnia-Herzegovina, La Verna chamber, in France, among others (Pellegrini and Ferreira 2012, Culver and Sket 2002, Faille et al. 2014). Unfortunately, for most show caves around

the world, the assessment of cave fauna has only occurred after the establishment of the tourism, so the pristine conditions of those caves, in most cases, remains unknown.

It worth stating that at La Verna chamber (France), cave tourism did not impose any negative impact on the troglobitic species, which include 18 endemic hypogean invertebrates (Faille et al. 2014). However, tourism at La Verna chamber has some particularities that allow such non-impact: 1) the huge chamber of the cave that prevents significant microclimatic changes, and also offers suitable micro-habitats for invertebrates to shelter themselves from disturbance and illumination; 2) Cave tourism occurs in limited duration during the year and limited spatial coverage, in accordance with cave size (Faille et al. 2014). On the other hand, in Lapa Nova Cave, touristic passages occur regardless of cave conduit size and species distribution, leading to severe disturbances to the cave fauna. At Vjetrenica Jama, although 60 obligate subterranean species co-exist with tourism, some stygobiont species have disappeared from cave areas because of leakage from batteries left in the cave by visitors (Culver and Sket 2002).

Conclusions

Lapa Nova cave revealed to be a quite peculiar system, not only regarding its dimensions and geology, but also as to the patterns existent in the associated fauna. Visitation at the cave probably altered the expected natural community distribution pattern, as has already been stated in Romania show caves studies (Moldovan et al. 2013), imposing a new organization of the communities, driven by the unstable conditions imposed by cave tourism. The need to conduct studies such as this in show caves is evident, although with longer temporal scales so that one can confirm the seasonal changes undergone by the cave communities. Furthermore, studies regarding sampling events before tourism establishment are especially important in order to evaluate population sizes, distributions and conditions to guarantee sustainable cave use.

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